The influence of size on body shape diversification across Indo-Pacific shore fishes

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Understanding the causes of body shape variability across the tree of life is one of the central issues surrounding the origins of biodiversity. One potential mechanism driving observed patterns of shape disparity is a strongly conserved relationship between size and shape. Conserved allometry has been shown to account for as much as 80% of shape variation in some vertebrate groups. Here, we quantify the amount of body shape disparity attributable to changes in body size across nearly 800 species of Indo-Pacific shore fishes using a phylogenetic framework to analyze 17 geometric landmarks positioned to capture general body shape and functionally significant features. In marked contrast to other vertebrate lineages, we find that changes in body size only explain 2.9% of the body shape variation across fishes, ranging from 3% to 50% within our 11 sampled families. We also find a slight but significant trend of decreasing rates of shape evolution with increasing size. Our results suggest that the influence of size on fish shape has largely been overwhelmed by lineage-specific patterns of diversification that have produced the modern landscape of highly diverse forms that we currently observe in nature.

KEY WORDS: Evolutionary allometry, geometric morphometrics, macroevolution, phylogenetic comparative methods.

Body size is a fundamental to an organism's biology. Size influences how organisms interact with their environment, how they fit into ecological communities, their longevity, and their reproductive output (Calder 2001). Variation in body size can provide a basis for differences in niche or mate choice that are important factors in speciation (Losos 1994; Nagel and Schluter 1998). Body size also underlies well-known macroevolutionary trends, including Cope's rule, where animal lineages evolve toward larger body sizes over time (LaBarbera 1989; Knouft and Page 2003; Heim et al. 2015) and Bergmann's rule, the trend toward larger body size in cold climates (Mayr 1956; Meiri and Dayan 2003). Additionally, it is well known that body shape often changes markedly with size within species over ontogeny. These and other patterns influencing body size may all have implications for the diversification of body shapes.

Species are often portrayed as having a static or characteristic shape, but in reality most display substantial shape change with body size (Gould 1966; Schmidt-Nielsen 1975; Calder 2001). This allometry is generally predictable within species (Cock 1966; Gould 1966) because of tight developmental integration and may also be strongly conserved between species (Klingenberg 1998; Voje et al. 2014). Assuming that the development of body shape is genetically controlled and heritable, natural selection acting on size will carry other traits along in a way that is predicted by the genetic covariance matrix (Bickel and Losos 2002). In this way, size may act as a line of least evolutionary resistance, a less constrained pathway for shape change (Schluter 1996; Marroig and Cheverud 2005), and selection on size may play a role in the creation of body shape variability (Pyron and Burbrink 2009).

In some cases, the influence of size on shape seems to play a major role. For example, due to tight integration between beak and braincase morphology in raptors, body size—not feeding ecology—was shown to account for almost 80% of beak shape variation (Bright et al. 2016). Additionally, a large component

of variability in mammal facial morphologies may be similarly driven by size variation, due to the covariation between the face and braincase (Cardini and Polly 2013). There is also the potential for changes in body size to produce shape changes that alter functional traits, even unlocking ecological opportunity (Pyron and Burbrink 2009). Although changes in shape have long been the focus of morphologists, the intrinsic covariation between structures, coupled with the tendency for shape to change with size, may allow evolution to create substantial functional and ecological diversity through changes in size.

In addition to facilitating shape variation, size may also influence the diversification of phenotypes through processes like size-dependent ecological opportunity. Different factors may influence rates of phenotypic diversification at opposite ends of the size spectrum. For example, smaller species may experience their environments as being more physically complex (Kulbicki et al. 2015), driving diversification of body form as it relates to microhabitat use. Conversely, if shapes of small individuals are strongly conserved but ontogenetic allometry diverges among species, then larger species will show greater differences in shape. Another way in which body size may influence shape diversification is through relationships with life history attributes like generation time and mass-specific metabolic rate, both of which influence rates of molecular evolution (Martin and Palumbi 1993; Calder 2001; Cooper and Purvis 2009). There is some evidence to suggest a correlation between rates of molecular and morphological evolution (Cooper and Purvis 2009; Sansalone et al. 2018). Thus, body size may indirectly alter rates of molecular evolution, which in turn could result in increased or decreased rates of morphological diversification at either end of the size continuum (Stanley 1979; Cooper and Purvis 2009).

In this study, we explore the role of body size in the evolution of Indo-Pacific shore fish body shapes. Ranging from the dwarf pygmy goby (9 mm) to the goliath grouper (2500 mm), shallow water shore fishes exhibit much of the body size disparity encompassed by all other vertebrate groups combined (Gillman 2007; Albert and Johnson 2012). Shore fishes also show remarkable body shape variability, including groups like highly elongate eels, deep-bodied and laterally compressed butterflyfishes, wide-bodied and dorsoventrally depressed flatheads, and a wide diversity of unique forms, such as those found in seahorses, frogfishes, and boxfishes (Claverie and Wainwright 2014; Price et al. 2015). But to what extent has body size evolution served as a mechanism for generating disparity of fish shapes? Although it is known that body shape and size often strongly covary within species, it is not known how much of acanthomorph body shape disparity is attributable to body size or how this relationship varies across families of fishes. Here, we address three questions: (1) How much of body shape disparity across shore fishes is explained by changes in body size? (2) How consistent is this overall relationship in 11 focal families? (3) What is the effect of body size on both disparity of fish shapes and the rate of body shape evolution across shore fishes?

Methods

DATA ACQUISITION AND PREPARATION

We subsampled a previously published landmark-based geometric morphometric dataset, which includes a single adult individual from each of 2939 species across 56 families of Indo-Pacific reef fishes (Claverie and Wainwright 2014). The dataset was generated on photographs in a repository of images taken by Dr. Jack Randall at the Bishop Museum and thus we cannot account for sexual dimorphism in our analysis. However, few examples of sexual shape dimorphism in tropical shore fishes have been reported, as most of the variation between sexes is in color pattern or some cases of fin elongation. What appears to be body shape sexual dimorphism in some labrids is ontogenetic body shape allometry coupled with size differences between individuals. Sexual dimorphism is found in some species of apogonids related to mouth brooding, but variation between sexes is much less than variation between species (Barnett and Bellwood 2005). Some male syngnathids have a brood pouch that is enlarged when the male is carrying eggs (Bell et al. 2003) but these were not sampled.

A set of 17 homologous landmarks were positioned to capture prominent features of body shape and functionally significant traits associated with both feeding and locomotion. We matched these morphometric data to the most comprehensive available time-calibrated phylogeny of fishes (Rabosky et al. 2013), yielding 791 species across 47 families with both morphometric and phylogenetic information for downstream analyses. Although the dataset captures considerable fish diversity, the sample is composed almost entirely of acanthomorph families intimately associated with coral reefs, with some additional representative lineages from nonacanthomorph clupeids (herrings, sardines, and their relatives) and peripherally reef-associated acanthomorph families (e.g., Belonidae, Hemiramphidae, Atherinidae, and Leiognathidae). The considerable fish diversity contained in this dataset allows us to assess the generality to which body size influences shape diversification across fishes under many different selective pressures. The phylogeny and morphometric data were pruned using the R packages *ape* (Paradis et al. 2004) and *geiger* (Harmon et al. 2008). All analyses for this study were conducted in the R statistical computing environment version 3.5.0. (R Core Team 2018).

Using *geomorph* version 3.0.6 (Adams et al. 2018), landmark coordinates were first aligned with a generalized Procrustes analysis (GPA), which rotates, scales, and translates landmark configurations. This step is used to isolate shape information, removing all other components of variation, including centroid

size. Centroid size is a measure of the amount of dispersion of landmarks around the centroid, or the center of the landmark configuration, and is thus a measure of overall size. It is calculated as the square root of the sum of squared distances from each landmark to the centroid (Bookstein 1997). Subsequent analyses were conducted on the multivariate Procrustes-aligned landmark coordinates (shape data) and univariate log-transformed centroid size (size data).

ALLOMETRIC ANALYSES

To assess the effects of size-related shape variation (allometry), we used the *procD.allometry* function implemented in *geomorph* (Adams et al. 2018). This function isolates and quantifies the common allometric component (CAC) across species, the aspect of shape variation most closely associated with changes in size (Mitteroecker et al. 2004). This allowed us to visualize the average allometric trend in body shape across our sample of species. We used a principal component analysis of the shape data (Procrustes coordinates) to visualize the dominant morphological trends across the dataset.

To quantify the fraction of body shape variation attributable to body size evolution, we performed a phylogenetic generalized least squares regression (PGLS) of shape on size, using the *geomorph* function *procD.pgls*. This function conducts a multivariate PGLS regression across all shape axes under a Brownian motion model of evolution, meaning it captures variation across all shape dimensions while accounting for phylogenetic nonindependence of species. We also conducted separate PGLS analyses of shape on size within each family for which we had >15% of species included in our dataset, resulting in 11 focal families (listed in Table 1). Species in each family were aligned using a GPA prior to the PGLS analysis. The statistical significance of all PGLS analyses were assessed with a permutation procedure (RRPP; 10 000 iterations). A benefit of using RRPP is that it estimates the effect size (Z-score) in standard deviates between the observed SS (sum of squares) and the sampling distribution of random SS, which facilitates comparisons both within and across analyses (Adams and Collyer 2018b).

FAMILY-LEVEL DISPARITY RELATIONSHIPS

To further explore factors that influence body shape disparity within clades, we examined the relationships between shape disparity, clade age, and rate of body shape evolution under Brownian motion for our 11 focal families. We Procrustes-aligned the shape data for each family and calculated morphological disparity using the *morphol.disparity* function in the R package *geomorph* (Adams et al. 2018) and evolutionary rates of multivariate body shape were estimated for each family using the *compare.evol.rates* function, also in *geomorph*.

BODY SHAPE DISPARITY AND BODY SIZE

To determine how body shape disparity varies with body size, we first discretized body size (log centroid size) into intervals of 0.05 units ("size bins"). Any size bin with fewer than 10 species was eliminated from the analysis due to the potential for unreliable disparity estimates due to low sample sizes. We then used the *morphol.disparity* function in *geomorph* to estimate the morphological disparity across all trait axes for species within each of the 21 remaining size bins. This function calculates disparity as the Procrustes variance, the sum of the diagonal elements of the group covariance matrix (Zelditch et al. 2004). Finally, we performed a nonphylogenetic linear regression between body shape disparity and body size using the procD.lm function in *geomorph*.

Each size bin was subjected to a modified jackknife procedure, in which one species was randomly removed from each size bin and the overall regression between morphological disparity and body size was recalculated for 1000 iterations. This was to ensure that disparity estimates were not strongly influenced by outlier species within a given size bin. We also performed the same procedure of estimating morphological disparity with different numbers of size bins, ranging from two to 30 bins to determine if our findings were sensitive to the binning process. Finally, we randomly reshuffled the size bins across the tips of the phylogeny to determine if there was phylogenetic signal to our size bins. In other words, species that are closely related may also be similar in body size and likely to fall into the same size category.

Under the Brownian motion process, trait disparity of a clade is a function of the Brownian rate parameter, σ^2, the age of the most recent common ancestor of the clade, and the average time between tips in the clade (O'Meara et al. 2006). Thus, disparity will increase with both clade age and under higher evolutionary rates (O'Meara et al. 2006). To investigate how these factors have influenced the relationship between body shape disparity and body size across the data set, we estimated the rate of body shape evolution for each size bin using a distance-based method of estimating multivariate evolutionary rates (Adams 2014).

Results
ALLOMETRIC ANALYSES

The CAC captured a tendency for smaller fishes to have larger eyes, smaller mouths, and deeper caudal peduncles, while larger fishes are more elongate with smaller eyes and a longer maxilla (Fig. 1). The dominant shape variation described by PC1 is primarily defined by changes in body depth and caudal peduncle length (Fig. 2). PC2 primarily represents changes in elongation of the body and fin bases. Elongate fishes with long dorsal and anal fins and a longer caudal peduncle cluster in the upper right

Table 1. Results from a PGLS of total body shape on size for families with >15% species sampled.

Family	R^2	F	Z	P	SS	No. species	% Sampled
Acanthuridae	0.50	31.63	3.98	<0.01	0.0141	34	41
Balistidae	0.06	0.96	0.15	0.45	0.0003	16	38
Carangidae	0.10	2.46	1.42	0.08	0.0018	24	16
Chaetodontidae	0.10	7.74	3.47	<0.01	0.0030	73	57
Holocentridae	0.18	3.06	2.45	<0.01	0.0003	16	19
Labridae	0.09	14.48	5.33	<0.01	0.0028	143	28
Lethrinidae	0.08	1.62	1.16	0.13	0.0002	20	53
Lutjanidae	0.14	4.85	2.72	<0.01	0.0013	33	30
Pomacanthidae	0.23	5.72	3.46	<0.01	0.0007	21	24
Pomacentridae	0.03	4.14	2.42	0.02	0.0021	127	33
Siganidae	0.17	3.17	2.13	0.01	0.0011	17	59

Note. *R*-squared values represent the amount of body shape variation explained by body size. *F*-statistics and *Z*-scores (effect size) are reported, along with the *P*-values and sum of squares (SS) for each PGLS. "No. species" is the number of species sampled per family and "% Sampled" is the percent of species assigned to each family that were sampled.
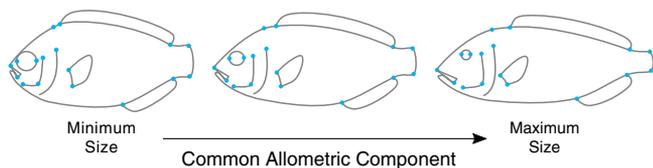

Figure 1. Illustration of the common allometric component (CAC), the shape changes across 791 species of Indo-Pacific shore fishes most closely associated with differences in body size. Blue points represent the landmark coordinates used to capture body shape variation. Left: smaller fishes; middle: mean shape across all fishes in the dataset; right: larger fishes.

region of morphospace, while deep-bodied species with shorter dorsal and anal fin attachments and a shorter caudal peduncle are relegated to the lower left region of this space (Fig. 2). Together, the first two principal components explain 56.5% of shape variation across fishes. No trend is immediately apparent between body size and the distribution of species in the space defined by PC1 and PC2 (Fig. 2).

The PGLS of body shape on size across all species is statistically significant with an R^2 of 0.029 ($P = 0.001$; $F = 23.82$; SS = 0.008). This means that 2.9% of shape variation across species is explained by body size. With a relatively large effect size ($Z = 6.58$), we find the influence of body size on shape disparity likely to be biologically significant and is greater than would be expected by chance (Collyer and Adams 2013; Adams and Collyer 2018b). However, the effect of body size on shape disparity varied sharply among the 11 focal families, ranging from an R^2 of 0.03 ($Z = 2.42$) in damselfishes (Pomacentridae) to 0.50 ($Z = 3.98$) in surgeonfishes (Acanthuridae; Table 1). Reported effect sizes (*Z*-scores) indicate that body size is a significant source of variation in body shape and that the null hypothesis of no re-

lationship between body size and shape can be rejected in many (but not all) of the 11 families studied here (excluding Balistidae, Carangidae, and Lethrinidae). These values further indicate that the effect of body size on shape disparity is quite variable across families. Overall, most families have low R^2 values, consistent with the result that body size does not strongly influence shape across fishes.

FAMILY-LEVEL DISPARITY RELATIONSHIPS
Even while controlling for phylogeny in the regressions, there is no significant relationship ($P > 0.05$) between body shape disparity and either rate of body shape evolution or clade age for the 11 families analyzed (Fig. 3). Shape disparity is not significantly correlated with rate of shape evolution ($P = 0.06$; $F = 4.7$; $Z = 1.17$), nor with clade age. We infer from these results that evolutionary rates likely vary enough within and between families to overshadow any effect of age.

We do, however, find some interesting differences among families. Surgeonfishes (family Acanthuridae) and jacks/pompanos (family Carangidae) possess some of the highest rates of both body shape evolution and body shape disparity across the families studied. We also find that damselfishes (family Pomacentridae) and angelfishes (family Pomacanthidae) have some of the lowest amounts of morphological disparity but vary considerably in rate of body shape evolution. These results largely agree with a previous study that also estimated body shape disparity in reef fish families (Price et al. 2015).

MORPHOLOGICAL DISPARITY AND BODY SIZE
There is a weak but significant increase in body shape disparity with body size across the dataset ($R^2 = 0.237$, $P = 0.019$; $F = 5.9$; $Z = 1.34$; Fig. 4). Based on the jackknife procedure,
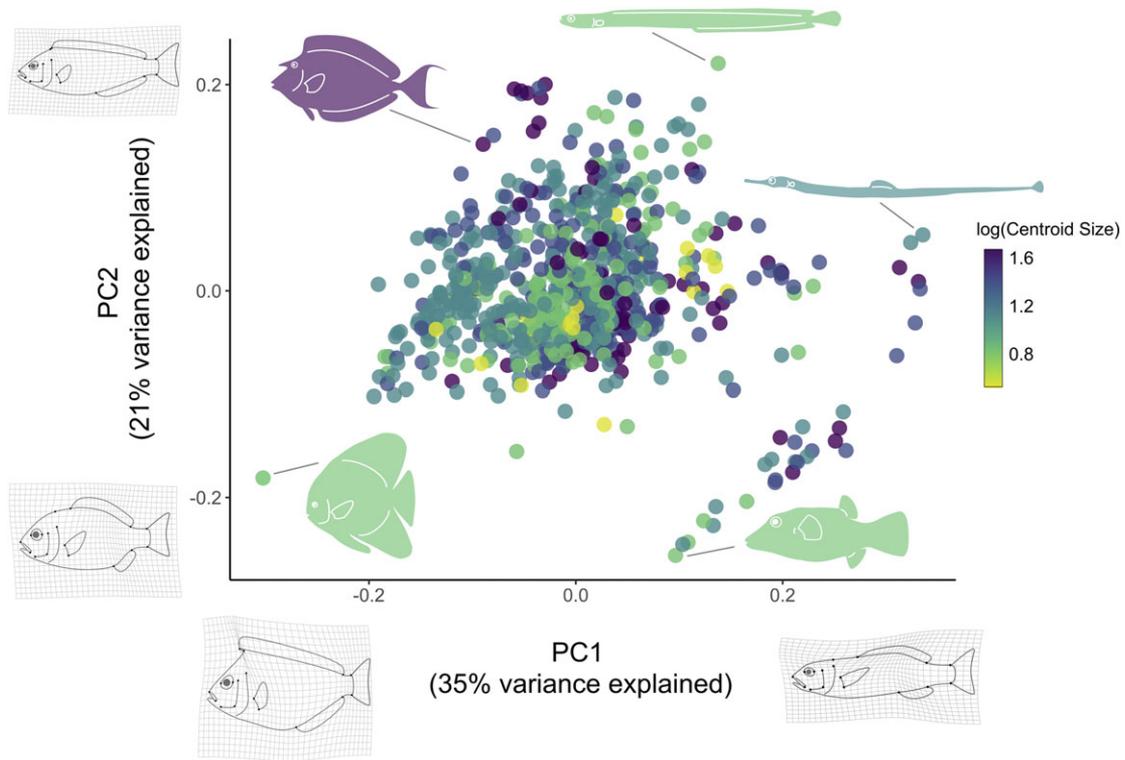
Figure 2. Plot of principal components 1 and 2, together accounting for 56.5% of total body shape variation across the dataset. Principal component analysis was conducted on the shape coordinates from 17 landmarks across 791 species. Point color corresponds to the log of centroid size in each species. Body shape variation along both PC 1 and 2 is shown as warp grids along the axes. Shapes of five species distributed across the morphospace are illustrated, clockwise from the upper left: *Naso unicornis*, *Gunnelichthys pleurotaenia*, *Corythoichthys intestinalis*, *Canthigaster janthinoptera*, and *Platax teira*.
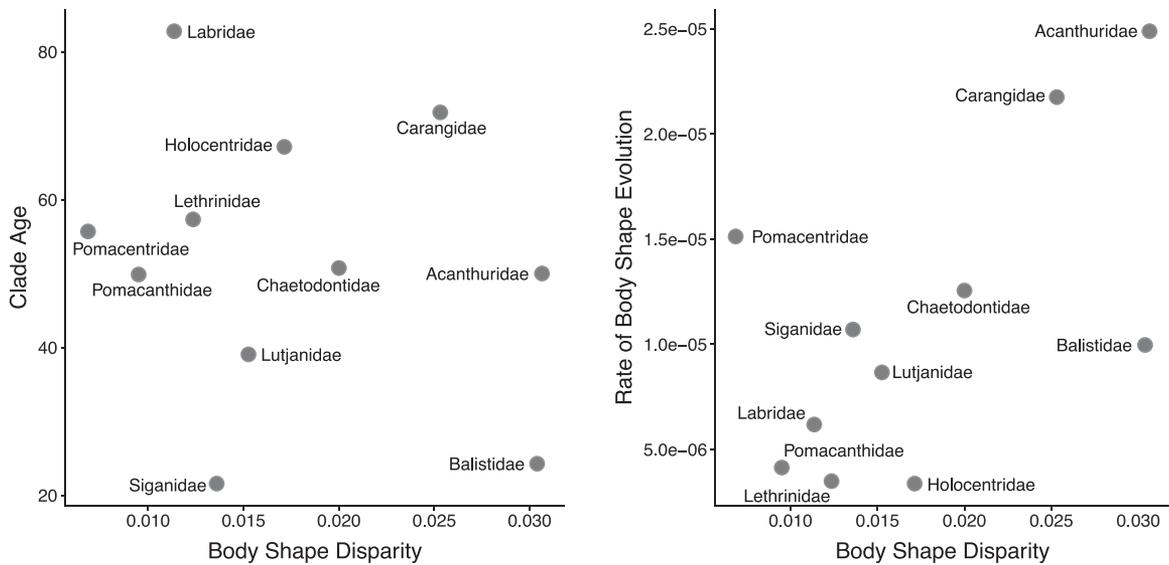

Figure 3. Relationships among the rates of body shape evolution, age of the most recent common ancestor of the clade in millions of years (clade age), and shape disparity (measured as Procrustes variance) for each family with >15% species sampled. No statistically significant relationships between morphological diversity and any of the response variables were recovered.
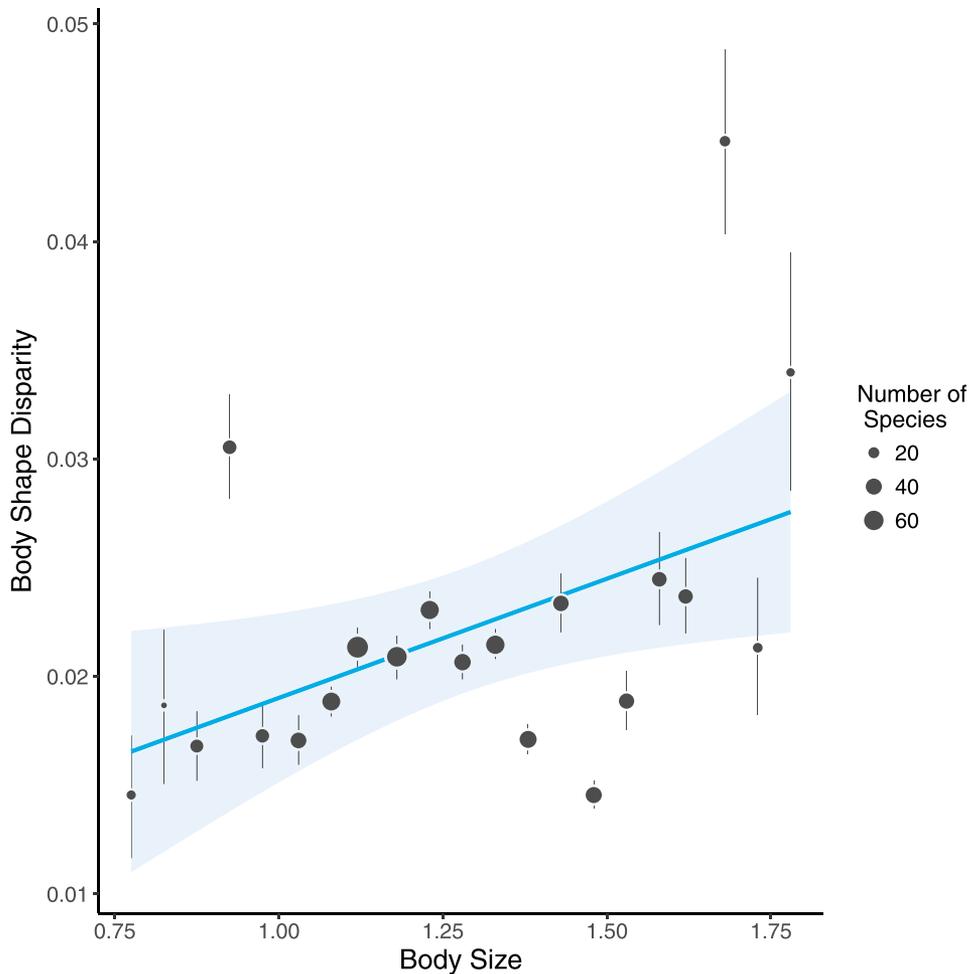
Figure 4. Relationship between body shape disparity, measured as Procrustes variance, and body size, measured as log-centroid size ($R^2 = 0.237$, $P = 0.019$). Point size corresponds to the number of species in a given body size interval. The shaded region is the 95% confidence interval around the regression line.

the regression line is relatively robust, ranging in effect size from 0.84 to 1.55 across the 1000 iterations and retaining a significant *P*-value ($P < 0.05$, $Z > 1.11$) in over 89% of the permutations (Supporting Information). We also find evidence that the pattern is only sensitive to the binning procedure with fewer than 12 size bins, at which point the regression is no longer significant ($P > 0.05$) between morphological disparity and body size (Supporting Information). Finally, we observe phylogenetic patterning in our size bins (supplementary materials) reflecting the tendency for body size to be relatively conserved; however, the majority of our size bins are not dominated by a single family of fishes (Supporting Information).

Although the slight increase in disparity with body size could potentially be caused by a similar effect of body size on rate of shape evolution, we found that rate of body shape evolution is weakly negatively correlated with body size ($R^2 = 0.338$, $P = 0.005$; $F = 9.72$; Fig. 5). Thus, species of larger body size tend to have slightly slower rates of shape evolution than smaller-bodied

species, although the effect size ($Z = 1.54$) indicates that this effect is weak.

Discussion

Strong interspecific allometry—phenotypic changes across species that are tightly associated with size (Gould 1966; Voje et al. 2014)—is often thought of as a constraint on shape, but it can also be viewed as a mechanism that promotes shape diversification. Typically, species grow along distinct allometric trajectories, the predictable path through morphospace reflected in the covariance between size and shape (Wilson 2013; Klingenberg 2016). One route for evolution is to follow these trajectories, such that shape changes predictably with changes in size (Klingenberg 1998; Pélabon et al. 2014; Voje et al. 2014). Alternatively, deviating from this fixed channel of shape variation can result in even greater phenotypic variability as other regions of morphospace are explored.
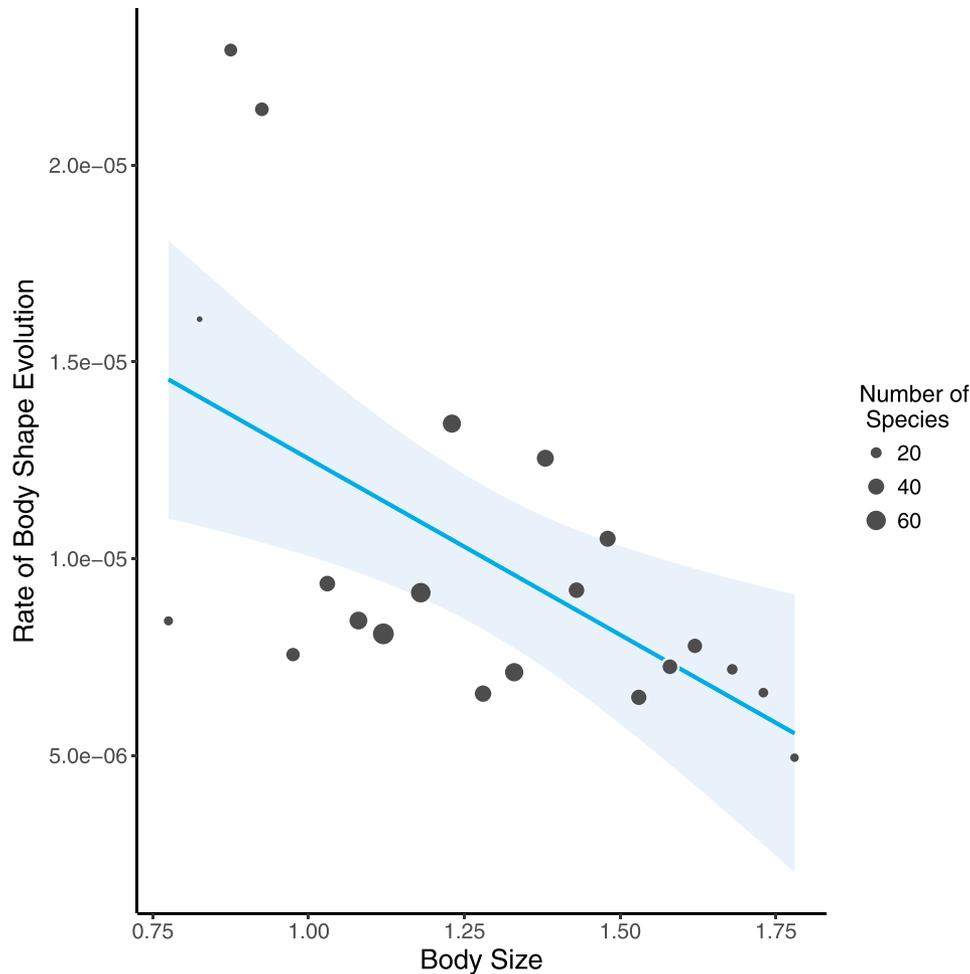
Figure 5. Relationship between rate of body shape evolution under Brownian motion and body size, measured as log centroid size ($R^2 = 0.338$, $P = 0.005$). Point size corresponds to the number of species in a given body size interval. The shaded region is the 95% confidence interval around the regression line.

We find that strong relationships that are known to exist between shape and size *within* species (Alberch et al. 1979; Voje et al. 2014) have not translated into a correspondingly strong relationship *across* fish species. Just 2.9% of body shape disparity was explained by body size. Indo-pacific shore fishes, therefore, appear to be a strong counter example to the tendency for body size to explain a large fraction of shape variation, as observed in clades of mammals and raptors. Although the literature only includes a few exemplar groups, previous studies have generally reported much higher fractions of interspecific variance in shape attributable to size (Cardini and Polly 2013; Bright et al. 2016). The implication of our result is that fish evolution has not been strongly tied to allometry, as the vast majority of shape evolution is independent of it. More generally, whatever set of conditions has led to size-related constraints on shape in other groups of organisms does not seem to have been a major factor in fish diversification.

We did find a substantial role of size in driving shape evolution in two families, Acanthuridae (50%) and Pomacanthidae (23%), and a statistically significant but smaller fraction in six other families. The importance of body size to shape variation in surgeonfishes (Acanthuridae) is related to differences in ecology between the two morphologically distinct subfamilies, Nasinae and Acanthurinae, that differ in both body size and shape, as well as ecology. Unicornfishes (subfamily Nasinae) tend to be larger, with a more elongate body form, and most are planktivores that swim well above the reef (Klanten et al. 2004). In contrast, most Acanthurinae are smaller, deep-bodied in shape (Friedman et al. 2016), and graze on benthic algae or detritus (Jones 1968). This diversity creates an axis of variation across the family between midwater species with an elongate body form and smaller, benthic feeders with a deep body shape. About 23% of angelfish (Pomacanthidae) shape disparity was explained by body size, but there are important differences between this group

and the acanthurids. First, disparity within this family is only about 25% of that found in acanthurids (Fig. 3A) and they rank in the bottom quartile of shore fish families in body shape disparity (Price et al. 2015). Most of the species included in our study are benthic feeders (Konow and Bellwood 2011) and have a distinctly deep-bodied shape, but pygmy angelfishes (*Centropyge*) are significantly smaller than the other genera in our study and tend to have a somewhat deeper body shape than other pomacanthids, thus creating a modest axis of body shape disparity that is correlated with body size. Overall, however, most of our focal families have remarkably low R^2 values, indicating that body size does not strongly constrain shape variation in fishes. Only three of 11 families showed no effect of size on shape variation (Table 1).

Another noteworthy clade-specific result includes our finding of both low disparity and low rates of body shape evolution in Labridae, a family with exceptionally high trophic diversity (Hiatt and Strasburg 1960; Randall 1967). Although other studies have revealed that this group shows tremendous variation in functional traits associated with feeding performance (Yamaoka 1978; Clifton and Motta 1998; Wainwright et al. 2004) and locomotion (Wainwright et al. 2002; Aiello et al. 2017), this does not seem to translate into variation in body shape.

Although it is unclear how fishes are able to overcome an often tight integration between size and shape, there are some significant differences between this study and others that have found strong relationships between the two on a macroevolutionary scale. This study included nearly 800 species of fishes, with an estimated age of the most recent common ancestor of 192 Ma (Rabosky et al. 2013), while others are focused at smaller and more recent taxonomic scales: 61 species of mammals and 147 raptor species, respectively (Cardini and Polly 2013; Bright et al. 2016). The deeper phylogenetic scope of this study introduces variation in the association between size and shape within families, potentially weakening the large-scale allometric pattern in favor of more phylogenetically local allometric relationships. This is evidenced by our finding of significant differences in the relationship between size and shape between families (ranging from an R^2 of 0.03 to 0.5). We also evaluated body shape as a whole, while the other studies solely focused on the shape of craniofacial elements, which have established allometric trends across a wide array of taxa (Claude et al. 2004; Slater and Van Valkenburgh 2009; Cardini and Polly 2013; Openshaw and Keogh 2014; Linde-Medina 2016). To our knowledge, this is the first study to examine the effects of evolutionary allometric constraints on whole body shape evolution.

FAMILY-LEVEL DISPARITY RELATIONSHIPS

At the family level, we find no relationship between body shape disparity and either rates of shape evolution or clade age. Evolutionary rates of body shape evolution are sufficiently variable as to overwhelm the expected tendency for disparity to accumulate in proportion to time. Macroevolutionary theory, as well as simulation studies, predictc a positive association between these three evolutionary factors (Gould and Eldredge 1997; Schluter 2000). However, empirical support for the expected relationship among disparity, age, and evolutionary rate is limited. Although a few studies do find relationships among these factors (Rabosky and Adams 2012; Zelditch et al. 2015), others find no correlation (Adams et al. 2009; Sherratt et al. 2014; Alhajeri and Steppan 2018). The inconsistency of this result indicates widespread heterogeneity in rates of phenotypic evolution.

The evolution of body shape in fishes probably has a complex set of drivers and it is unclear if there are any universal ecological or biological determinates for patterns of body shape variation. Particular families appear to diversify along different niche axes (body size, diet, habitat, etc.), ultimately leading to shape disparity within the clade. For example, acanthurids and balistids harbor some of the largest shape disparity of the families studied here (Fig. 3). Diversification in these families has been demonstrated to be partially driven by adaptations to use of open water habitats as opposed to moving around in close proximity to reef structures (Dornburg et al. 2011; Friedman et al. 2016). A similar relationship may occur in carangids with open water fish possibly being characterized by more slender, elongate body shape and species that swim in close proximity to the substrate tending to be more deep-bodied (Frederich et al. 2016).

Damselfishes (Pomacentridae) had among of the lowest body shape disparity in our group of focal families but show high rates of body shape evolution, consistent with their reputation for transitioning between three trophic guilds at a high rate (Cooper and Westneat 2009; Frédérich et al. 2013). They occupy some of the most densely packed regions of morphospace (Price et al. 2015) and show that high rates of body shape evolution need not be associated with parallel achievement of high ecological or morphological diversity. Combined with our findings here that neither clade age nor rate of body shape evolution is correlated with shape disparity, perhaps the drivers of diversification are clade dependent.

MORPHOLOGICAL DISPARITY AND BODY SIZE

Allometry is a ubiquitous feature of organismal growth and development. As organisms increase in size, traits may become more disparate between species (Frankino et al. 2005; Urošević et al. 2013). This phenomenon may provide an explanation for our finding of increased body shape disparity in larger fishes. Additionally, recent research has focused on the extent to which allometric growth parameters themselves can evolve. The concept of allometric differentiation driving morphological evolution has been explored in marine snails (Hollander et al.

2006) and rodents (Wilson and Sanchez-Villagra 2010; Wilson 2013). Recent studies have reinforced the idea that allometries evolve and these changes can have an adaptive basis. Ecological and functional aspects of organismal life, such as diet or habitat, can result in species-specific changes to allometric trajectories (Adams and Nistri 2010; Wilson 2013). Although many of these studies have emphasized changing allometries resulting in morphological variability on a smaller taxonomic scale than that considered here, they all acknowledge the potential for these allometric effects to result in large-scale macroevolutionary patterns (Wilson and Sanchez-Villagra 2010; Urošević et al. 2013). The extent to which the evolution of allometric trajectories has indeed influenced macroevolutionary patterns remains largely unexplored and would benefit from future work.

Various authors have discussed that both extremely small (Munday and Jones 1998) and extremely large fishes (Albert and Johnson 2012) may encounter reduced ecological opportunity in the form of fewer options for prey and microhabitat, although the most prevalent proposition is that niche opportunities are greater for smaller fishes (Rüber et al. 2007). Our study indicates that, at least among shore fishes, there seems to be little evidence of constraints on body shape diversification at either body size extreme. We found a tendency for smaller fishes to experience faster body shape evolution (Fig. 5) but high variation in evolutionary rate both within size groups and across fishes makes this a relatively weak effect. For example, Serranidae, including many very large species, and Gobiidae, with high species richness and small body size, both have high body shape disparity (Price et al. 2015). Whether our findings for rates of body shape evolution reflect similar rates of ecological evolution remains to be seen.

An interesting trend that deserves further explanation is the negative relationship between rate of shape evolution and body size. This finding cannot be explained by the phylogenetic patterning of the size bins, as we have corrected for phylogeny by calculating the rate of Brownian motion evolution, which is effectively a time-corrected measure of shape disparity. We find larger species of fishes have slower rates of shape evolution than smaller species. Similar studies have found the directionality of the trend between rates of shape evolution and body size varies with clade, and in some cases, the relationship is nonlinear with the highest rates of morphological evolution at both size extremes (Cooper and Purvis 2009). The negative relationship between body size and rates of shape evolution may reflect differences in life history between species of different body sizes. Factors such as generation time, number of offspring, lifespan, and metabolic rate are known to scale with body size such that the combination of these traits may result in decreased rates of evolution in larger species (Martin and Palumbi 1993; Bromham et al. 1996; Gillooly et al.

2005; Bromham 2011; Sibly et al. 2015). Similarly, there is substantial evidence for a negative correlation between body size and rates of molecular evolution in reptiles (Bromham 2002), mammals (Bromham 2011), and scombrid fishes (Qiu et al. 2014). Although rates of molecular and morphological evolution are not equivalent, as all morphological traits must have some genetic basis, there may be at least a complex connection between them. While there is some evidence linking the two (Omland 1997), the relationship remains somewhat tenuous (Bromham et al. 2002).

We acknowledge that the discovery of increased shape disparity, yet lower rates of shape evolution at larger body sizes, is somewhat paradoxical. Although we offer some explanation, the process behind our findings remains undetermined. This result may be partially due to our underlying evolutionary model, Brownian motion. One of the principal limitations in modern macroevolutionary biology is the application of phylogenetic comparative methods to high-dimensional data. Recently, it has come to light that multivariate phylogenetic comparative methods, especially comparisons between evolutionary models, are unreliable and highly susceptible to model misspecification (Adams and Collyer 2018a). In this study, we have attempted to control—at least in part—for the phylogenetic nonindependence of species while remaining cautious of methodological limitations. Therefore, we assume Brownian motion, the simplest model of evolution, as the underlying evolutionary process for our phylogenetic analyses. In all likelihood trait evolution is probably both bounded and more complex, with varying rates of evolution both across lineages and through time. However, until appropriate comparative methods for dealing with multivariate data are developed, we remain conservative in our methodological approach.

In conclusion, we highlight that body shape diversification within fishes is largely independent of body size. We also show that the relationship between size and shape is clade specific. Although the rate of morphological evolution is slower in larger fishes, larger-bodied species nevertheless tend to harbor more shape disparity than smaller species. Fish body shapes are highly diverse, and our results provide the first evidence that size has not been a significant constraint on body form diversification across the evolution of spiny-rayed fishes.

DATA ARCHIVING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

LITERATURE CITED

Adams, D. C. 2014. Quantifying and comparing phylogenetic evolutionary rates for shape and other high-dimensional phenotypic data. Syst. Biol. 63:166–177.

Adams, D. C., C. M. Berns, K. H. Kozak, and J. J. Wiens. 2009. Are rates of species diversification correlated with rates of morphological evolution? Proc. R. Soc. Lond. B Biol. Sci. 276:2729–2738.

Adams, D. C., and A. Nistri. 2010. Ontogenetic convergence and evolution of foot morphology in European cave salamanders (family: Plethodontidae). BMC Evol. Biol. 10:1–10.

Adams, D. C., and M. L. Collyer. 2018a. Multivariate phylogenetic comparative methods: evaluations, comparisons, and recommendations. Syst. Biol. 67:14–31.

———. 2018b. Phylogenetic ANOVA: group-clade aggregation, biological challenges, and a refined permutation procedure. Evolution 72:1204–1215.

Adams, D. C., M. L. Collyer, and A. Kaliontzopoulou. 2018. Geomorph: Software for geometric morphometric analyses. R package version 3.0.6. https://cran.r-project.org/package=geomorph.

Aiello, B. R., M. W. Westneat, and M. E. Hale. 2017. Mechanosensation is evolutionarily tuned to locomotor mechanics. Proc. Natl. Acad. Sci. USA 114:4459–4464.

Alberch, P., S. J. Gould, G. F. Oster, and D. B. Wake. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Albert, J. S., and D. M. Johnson. 2012. Diversity and evolution of body size in fishes. Evol. Biol. 39:324–340.

Alhajeri, B. H., and S. J. Steppan. 2018. Disparity and evolutionary rate do not explain diversity patterns in Muroid rodents (Rodentia: Muroidea). Evol. Biol. 45:324–344.

Barnett, A., and D. R. Bellwood. 2005. Sexual dimorphism in the buccal cavity of paternal mouthbrooding cardinalfishes (Pisces: Apogonidae). Mar. Biol. 148:205–212.

Bell, E. M., J. F. Lockyear, and J. M. McPherson. 2003. First field studies of an endangered South African seahorse, Hippocampus capensis. Environ. Biol Fishes 67:35–46.

Bickel, R., and J. B. Losos. 2002. Patterns of morphological variation and correlates of habitat use in Chamaleons. Biol. J. Linn. Soc. 76:91–103.

Bookstein, F. L. 1997. Morphometric tools for landmark data: geometry and biology. Cambridge Univ. Press, Cambridge, U.K.

Bright, J. A., J. Marugán-Lobón, S. N. Cobb, and E. J. Rayfield. 2016. The shapes of bird beaks are highly controlled by nondietary factors. Proc. Natl. Acad. Sci. USA 113:5352–5357.

Bromham, L. 2002. Molecular clocks in reptiles: life histories influences rate of molecular evolution. Mol. Biol. Evol. 19:302–309.

———. 2011. The genome as a life-history character: why rate of molecular evolution varies between mammal species. Philos. Trans. R. Soc. B Biol. Sci. 366:2503–2513.

Bromham, L., A. Rambaut, and P. H. Harvey. 1996. Determinants of rate variation in mammalian DNA sequence evolution. J. Mol. Evol. 43:610–621.

Bromham, L., M. Woolfit, M. S. Y. Lee, and A. Rambaut. 2002. Testing the relationship between morphological and molecular rates of change along phylogenies. Evolution 56:1921–1930.

Calder, W. A. 2001. Ecological Consequences of Body Size. in eLS, Ed. John Wiley & Sons, Ltd, Chichester, U.K. https://doi.org/10.1038/npg.els.0003208.

Cardini, A., and P. D. Polly. 2013. Larger mammals have longer faces because of size-related constraints on skull form. Nat. Commun. 4:1–7.

Claude, J., P. C. H. Pritchard, H. Tong, E. Paradis, and J. C. Auffray. 2004. Ecological correlates and evolutionary divergence in the skull of turtles: a geometric morphometric assessment. Syst. Biol. 53:933–948.

Claverie, T., and P. C. Wainwright. 2014. A morphospace for reef fishes: elongation is the dominant axis of body shape evolution. PLoS One 9:e112732.

Clifton, K. B., and P. J. Motta. 1998. Feeding morphology, diet, and eco-morphological relationships among five Caribbean labrids (Teleostei, Labdridae). 1Copeia 1998:953–966.

Cock, A. G. 1966. Genetical aspects of metrical growth and form in animals. Q. Rev. Biol. 41:131–190.

Collyer, M. L., and D. C. Adams. 2013. Phenotypic trajectory analysis: comparison of shape change patterns in evolution and ecology. Hystrix 24:75–83.

Cooper, N., and A. Purvis. 2009. What factors shape rates of phenotypic evolution? A comparative study of cranial morphology of four mammalian clades. J. Evol. Biol. 22:1024–1035.

Cooper, W. J., and M. W. Westneat. 2009. Form and function of damselfish skulls: rapid and repeated evolution into a limited number of trophic niches. BMC Evol. Biol. 9:1–17.

Dornburg, A., B. L. Sidlauskas, F. Santini, L. Sorenson, T. J. Near, and M. E. Alfaro. 2011. The influence of an innovative locomotor strategy on the phenotypic diversification of triggerfish (family: Balistidae). Evolution 65:1912–1926.

Frankino, W. A., B. J. Zwaan, D. L. Stern, and P. M. Brakefield. 2005. Natural selection and developmental constraints in the evolution of allometries. Science 307:718–720.

Frédérich, B., L. Sorenson, F. Santini, G. J. Slater, and M. E. Alfaro. 2013. Iterative Ecological Radiation and Convergence during the Evolutionary History of Damselfishes (Pomacentridae). Am. Nat. 181:94–113.

Frédérich, B., G. Marrama, G. Carnevale, and F. Santini. 2016. Non-reef environments impact the diversification of extant jacks, remoras and allies (Carangoidei, Percomorpha). Proc. R. Soc. Lond. B Biol. Sci. 283:20161556.

Friedman, S. T., S. A. Price, A. S. Hoey, and P. C. Wainwright. 2016. Ecomorphological convergence in planktivorous surgeonfishes. J. Evol. Biol. 29:965–978.

Gillman, M. P. 2007. Evolutionary dynamics of vertebrate body mass range. Evolution 61:685–693.

Gillooly, J. F., A. P. Allen, G. B. West, and J. H. Brown. 2005. The rate of DNA evolution: effects of body size and temperature on the molecular clock. PNAS 102:140–5.

Gould, S. J. 1966. Allometry and size in ontogeny and phylogeny. Biol. Rev. 41:587–638.

Gould, S. J., and N. Eldredge. 1997. The tempo and mode of evolution reconsidered. Paleobiology 3:115–151.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.

Heim, N. A., M. L. Knope, E. K. Schaal, S. C. Wang, and J. L. Payne. 2015. Cope's rule in the evolution of marine animals. Science 347:867–870.

Hiatt, R. W., and D. W. Strasburg. 1960. Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol. Monogr. 30:65–127.

Hollander, J., D. C. Adams, and K. Johannesson. 2006. Evolution of adaptation through allometric shifts in a marine snail. Evolution 60:2490–2497.

Jones, R. S. 1968. Ecological relationships in Hawaiian and Johnston Island Acanthuridae (surgeonfishes). Micronesica, 4:309–361.

Klanten, S. O., L. Van Herwerden, J. H. Choat, and D. Blair. 2004. Patterns of lineage diversification in the genus Naso (Acanthuridae). Mol. Phylogenet. Evol. 32:221–235.

Klingenberg, C. P. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. Biol. Rev. 73:79–123.

———. 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. Dev. Genes Evol. 226:113–137.

Knouft, J. H., and L. M. Page. 2003. The evolution of body size in extant groups of North American freshwater fishes: speciation, size distributions, and Cope's rule. Am. Nat. 161:413–421.

Konow, N., and D. R. Bellwood. 2011. Evolution of high trophic diversity based on limited functional disparity in the feeding apparatus of marine angelfishes (f. Pomacanthidae). PLoS ONE 6:e24113.

Kulbicki, M., V. Parravicini, and D. Mouillot. 2015. Patterns and processes in reef fish body size. Pp. 104–115 in C. Mora, ed. Ecology of fishes on coral reefs. Cambridge Univ. Press, Cambridge, U.K.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. Annu. Rev. Ecol. Syst. 20:97–117.

Linde-Medina, M. 2016. Testing the cranial evolutionary allometric "rule" in Galliformes. J. Evol. Biol. 29:1873–1878.

Losos, J. B. 1994. Integrative approaches to evolutionary ecology: Anolis lizards as model systems. Annu. Rev. Ecol. Syst. 25:467–493.

Marroig, G., and J. M. Cheverud. 2005. Size as a line of least evolutionary resistence: diet and adatative morphological radiation in New World monkeys. Evolution 59:1128–1142.

Martin, A. P., and S. R. Palumbi. 1993. Body size, metabolic rate, generation time, and the molecular clock. PNAS 90:4087–4091.

Mayr, E. 1956. Geographical character gradients and climatic adaptation. Evolution 10:105–108.

Meiri, S., and T. Dayan. 2003. On the validity of Bergmann's rule. J. Biogeogr. 30:331–351.

Mitteroecker, P., P. Gunz, M. Bernhard, K. Schaefer, and F. L. Bookstein. 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. J. Hum. Evol. 46:679–698.

Munday, P. L., and G. P. Jones. 1998. Ecological implications of small body size among coral-reef fishes. Oceanogr. Mar. Biol. 36:373–411.

Nagel, L., and D. Schluter. 1998. Body size, natural selection, and speciation in sticklebacks. Evolution 52:209–218.

O'Meara, B. C., C. Ané, M. J. Sanderson, P. C. Wainwright, and C. Ane. 2006. Testing for different rates of continuous trait evolution using likelihood. Evolution 60:922–933.

Omland, K. E. 1997. Correlated rates of molecular and morphological evolution. Evolution 51:1381–1393.

Openshaw, G. H., and J. S. Keogh. 2014. Head shape evolution in monitor lizards (Varanus): interactions between extreme size disparity, phylogeny and ecology. J. Evol. Biol. 27:363–373.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Pélabon, C., C. Firmat, G. H. Bolstad, K. L. Voje, D. Houle, J. Cassara, A. Le Rouzic, and T. F. Hansen. 2014. Evolution of morphological allometry. Ann. N. Y. Acad. Sci. 1320:58–75.

Price, S. A., T. Claverie, T. J. Near, and P. C. Wainwright. 2015. Phylogenetic insights into the history and diversification of fishes on reefs. Coral Reefs 34:997–1009.

Pyron, R. A., and F. T. Burbrink. 2009. Body size as a primary determinant of ecomorphological diversification and the evolution of mimicry in the lampropeltinine snakes (Serpentes: Colubridae). J. Evol. Biol. 22:2057–2067.

Qiu, F., A. Kitchen, J. G. Burleigh, and M. M. Miyamoto. 2014. Scombroid fishes provide novel insights into the trait/rate associations of molecular evolution. J. Mol. Evol. 78:338–348.

Rabosky, D. L., and D. C. Adams. 2012. Rates of morphological evolution are correlated with species richness in salamanders. Evolution 66:1807–1818.

Rabosky, D. L., F. Santini, J. M. Eastman, S. A. Smith, B. L. Sidlauskas, J. Chang, and M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Commun. 4:1958.

Randall, J. E. 1967. Food habits of reef fishes of the West Indies. Stud. Trop. Oceanogr. 5:665–847.

Rüber, L., M. Kottelat, H. H. Tan, P. K. L. Ng, and R. Britz. 2007. Evolution of miniaturization and the phylogenetic position of Paedocypris, comprising the world's smallest vertebrate. BMC Evol. Biol. 7:1–10.

Sansalone, G., P. Colangelo, T. Kotsakis, A. Loy, R. Castiglia, A. A. Bannikova, E. D. Zemlemerova, and P. Piras. 2018. Influence of evolutionary allometry on rates of morphological evolution and disparity in strictly subterranean moles (Talpinae, Talpidae, Lipotyphla, Mammalia). J. Mamm. Evol. 25:1–14.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.

Schmidt-Nielsen, K. 1975. Scaling in biology: the consequences of size. J. Exp. Zool. 194:287–307.

Sherratt, E., D. J. Gower, C. P. Klingenberg, and M. Wilkinson. 2014. Evolution of cranial shape in Caecilians (Amphibia: Gymnophiona). Evol. Biol. 41:528–545.

Sibly, R. M., J. Baker, J. M. Grady, S. M. Luna, A. Kodric-Brown, C. Venditti, and J. H. Brown. 2015. Fundamental insights into ontogenetic growth from theory and fish. Proc. Natl. Acad. Sci. USA 112:13934–13939.

Slater, G. J., and B. Van Valkenburgh. 2009. Allometry and performance: the evolution of skull form and function in felids. J. Evol. Biol. 22:2278–2287.

Stanley, S. M. 1979. Macroevolution: pattern and process. W.H. Freeman and Company, San Francisco, CA.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Urošević, A., K. Ljubisavljević, and A. Ivanović. 2013. Patterns of cranial ontogeny in lacertid lizards: morphological and allometric disparity. J. Evol. Biol. 26:399–415.

Voje, K. L., T. F. Hansen, C. K. Egset, G. H. Bolstad, and C. Pélabon. 2014. Allometric constraints and the evolution of allometry. Evolution 68:866–885.

Wainwright, P. C., D. R. Bellwood, and M. W. Westneat. 2002. Ecomorphology of locomotion in labrid fishes. Environ. Biol. Fishes 65:47–62.

Wainwright, P. C., D. R. Bellwood, M. W. Westneat, J. R. Grubich, and A. S. Hoey. 2004. A functional morphospace for the skull of labrid fishes: Patterns of diversity in a complex biomechanical system. Biol. J. Linn. Soc. 82:1–25.

Wilson, L. A. B. 2013. Allometric disparity in rodent evolution. Ecology and Evolution 3:971–984.

Wilson, L. A. B., and M. R. Sanchez-Villagra. 2010. Diversity trends and their ontogenetic basis: an exploration of allometric disparity in rodents. Proc. R. Soc. Lond. B Biol. Sci. 277:1227–1234.

Yamaoka, K. 1978. Pharyngeal jaw structure in labrid fish. Publ. Seto Mar. Biol. Lab. 24:409–426.

Zelditch, M. L., J. Li, L. A. P. Tran, and D. L. Swiderski. 2015. Relationships of diversity, disparity, and their evolutionary rates in squirrels (Sciuridae). Evolution 69:1284–1300.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Academic Press, Cambridge, MA.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Relationship between body shape disparity, measured as Procrustes variance, and body size, measured as log-centroid size (blue line: $R^2 = 0.237$, $P = 0.019$).

Figure S2. Relationship between body shape disparity, measured as Procrustes variance, and body size, measured as log-centroid size (black line: $R^2 = 0.237$, $P = 0.019$; $F = 5.9$; $Z = 1.34$).

Figure S3. Histogram of 1000 recalculated regression slopes of body shape disparity versus body size.

Table S1. Table of the percentage of each bin composition by family.

Table S2. Table of the 791 species (and corresponding family names) with both morphometric and phylogenetic data used in this study.